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# Thyroxine stimulates transglutaminase activity in articular chondrocytes

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## Summary

*Objectives*: Thyroid hormones induce features of the hypertrophic phenotype in mature articular chondrocytes as well as in growth plate chondrocytes. Hypertrophic chondrocytes are responsible for extracellular matrix mineralization, with formation of bone mineral in growth plate cartilage and pathologic calcium crystals in aging articular cartilage. Elevated activity levels of the two transglutaminase (Tgase) enzymes (type II Tgase and Factor XIIIA (FXIIIA)) have recently been described as additional features of hypertrophic growth plate chondrocytes. Because Tgases may participate in pathologic mineralization in aging cartilage, we explored the effects of thyroid hormones on Tgase activity in articular chondrocytes.

*Methods*: Adult porcine articular chondrocytes were incubated with or without 250–750 nM L-thyroxine (T4) or 10–100 nM 3,3′,5-triiodothyronine (T3). Tgase activity was measured with a standard radiometric assay. The effects of thyroid hormones on protein and mRNA levels of type II Tgase and FXIIIA were determined. As Tgase activity can be stimulated by proteases, endoproteinase levels were also measured. The mechanisms of these effects were explored.

*Results*: T4 (750 nM) or T3 (100 nM) stimulated Tgase activity by twofold in articular chondrocytes at 4 h and increased the percentage of Tgase activity in the extracellular matrix. Chondrocytes rapidly converted T4 to T3, but the time course suggests similar mechanisms for T4 and T3. T4-induced Tgase activity was suppressed with cycloheximide and protein kinase C inhibitors. The effects of T4 on type II Tgase and FXIIIA levels were modest, but T4 strongly induced endoproteinase activity in chondrocytes.

*Conclusions*: We report in this study that thyroid hormones increase Tgase activity in articular chondrocytes via a non-genomic mechanism, which may involve increased endoproteinase secretion.


*Key words:* Articular cartilage, Calcium pyrophosphate dihydrate, Thyroid hormones, Transglutaminase.

## Introduction

The transglutaminase (Tgase) enzyme family comprises at least nine different enzymes that form ε-(γ-glutamyl) lysine bonds between or within proteins[1]. Recent evidence supports the hypothesis that Tgases participate in the pathologic cartilage matrix mineralization that often accompanies degenerative arthritis, and that results in apatite and calcium pyrophosphate dihydrate (CPPD) crystal formation[2]. Tgase activity levels dramatically increase with age in articular chondrocytes, mirroring the increased prevalence of clinical degenerative arthritis and articular calcium crystal deposition[3]. Increased Tgase activity correlates with elevated protein levels of the two forms of Tgase present in articular cartilage: Factor XIIIA (FXIIIA) and type II Tgase[3,4]. Tgase enzymes are present in the pericellular matrix of osteoarthritic chondrocytes at potential sites of pathologic calcium crystal formation[5]. *In vitro*, Tgase enzymes participate in the formation of apatite crystals in cartilage[2], while Tgase inhibitors suppress CPPD crystal formation[6].

Little is known about the regulation of Tgase activity in articular cartilage. Interleukin 1 remains the only identified stimulant of chondrocyte Tgase activity[2]. However, proteases, including trypsin and thrombin, are potent stimulants of Tgase action in other settings[7,8]. These enzymes can directly activate FXIIIA through proteolytic cleavage of an activation peptide[8], and can upregulate enzyme synthesis[7].

Although little is known about the causes of pathologic mineralization in degenerative arthritis, there is ample evidence to suggest that the chondrocytes responsible for this activity have many features in common with the hypertrophic chondrocytes that mineralize extracellular matrix in growth plate cartilage. Histologic studies show abnormally large chondrocytes near CPPD crystal deposits[9]. Aging articular chondrocytes and hypertrophic chondrocytes both elaborate matrix vesicles, which may directly participate in mineralization[10,11]. Thyroid hormones induce markers of the hypertrophic phenotype, such as type X collagen, and alkaline phosphatase, in both growth plate[12] and aging articular chondrocytes[13], and are capable of inducing matrix mineralization in cultured chondrocytes under serum-free conditions[12].

Recently, elevated levels of Tgase proteins have been described as an additional feature of the hypertrophic

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phenotype of growth plate chondrocytes[14,15]. These enzymes likely alter extracellular matrix proteins so as to facilitate mineral deposition[16]. In this study, we sought to determine if thyroid hormones increase Tgase activity in articular chondrocytes, and to explore the mechanism of this effect. We show in this study that L-thyroxine (T4) and 3,3′ 5-triiodothyronine (T3) markedly stimulate Tgase activity in articular chondrocytes. The mechanism is likely non-genomic, is protein kinase C-dependent and involves increases in endoproteinase activity.

## Materials and methods

### CHONDROCYTE CULTURES

Normal porcine articular cartilage was removed from the femoral and patellar surfaces of 3–5 years old animals (Johnsonville Foods Company, Watertown, WI). Chondrocytes were enzymatically isolated as previously described and plated at $4\times10^5$ cells/cm$^2$ in Dulbecco's Modified Eagle's Medium (DMEM; Mediatech, Herndon, VA) with 10% fetal calf serum[13]. Within 2–3 days of plating, the culture medium was changed to DMEM with 0.35 mg/ml bovine serum albumin (BSA) for 24 h. Experiments were performed in serum-free DMEM with 0.35 mg/ml BSA and 10–100 nM T3 or 250–750 nM T4 (Sigma Chemical Co., St. Louis, MO). These concentrations had previously been shown to be effective in stimulating markers of hypertrophy in articular chondrocytes[13]. For some experiments, whole cell layers were obtained by scraping chondrocytes and matrix off culture plates. Extracellular matrix was isolated from parallel cultures using sodium deoxycholate[17]. In other experiments, cell lysates were obtained by exposure of chondrocytes to M-PER™ (Pierce, Rockford, IL). Cytosol and membrane fractions were isolated by centrifuging cell lysates for 5 min at 6000×*g* to remove debris, and then for 30 min at 48 000×*g*[4].

### INHIBITORS

The non-glycosidic indolocarbazole (NGIC; 10 nM; Calbiochem) was used to block protein kinase C activity. Actinomycin D (100 nM; Sigma Chemical Co.) and cycloheximide (500 nM; Sigma Chemical Co.) were used to inhibit mRNA production and protein synthesis, respectively. α1 antitrypsin (50 µg/ml; ICN, Costa Mesa, CA) was used as an endoproteinase inhibitor.

### TGASE ASSAY

A standard radiometric assay based on the ability of Tgase to incorporate ($^3$H)putrescine into casein was used to measure Tgase activity in cell layers, cell fractions and extracellular matrix[3]. Results were expressed as Units (picomoles casein incorporated per 30 min) corrected for the protein content of the sample.

### T3 ASSAY

Chondrocytes were incubated with no additives or 3 ng/ml T3 or 10 ng/ml T4 for 4 h. T3 levels were measured in the cell layer using a commercial enzyme immunoassay (EIA) test kit for T3 according to manufacturer's directions (ICN Pharmaceuticals, Orangeburg, NY).

### WESTERN BLOTTING

Chondrocytes were lysed with M-PER™ after incubation for 4 h with or without thyroid hormones. Volumes of cell lysate normalized for protein content were loaded onto 10% NuPage® Bis-Tris gels (Invitrogen, Carlsbad, CA). Proteins were transferred to poly(vinylidene) difluoride (PVDF) membranes. After blocking for 1 h in Tris-buffered saline-Nonidet P40-5% skim milk, they were incubated with 1:250 dilution of goat anti-type II Tgase (Upstate Biotechnology, Lake Placid, NY) or 1:1000 dilution of rabbit anti-FXIII-A subunit (Calbiochem). There is no cross-reactivity between these antibodies, although both forms of Tgase are of similar molecular weight in chondrocytes[4]. The appropriate Immunopure® peroxidase-conjugated secondary antibody was used. The blot was developed with Supersignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL). For protein kinase C translocation experiments, chondrocytes were fractionated into cytosolic and membrane compartments[4], and blotted with a 1:500 dilution of rabbit anti-protein kinase C αβγ (Life Technologies, Gaithersburg, MD).

### FXIIIA ELISA

This assay accurately measures total FXIIIA protein levels and is based on a protocol from Affinity Biologicals (Ontario, Canada). Costar high-binding plates were coated with a 1:100 dilution of rabbit anticoagulation FXIIIA-subunit (100 µl/well; Calbiochem) in coating buffer (50 mM carbonate–bicarbonate, pH 9.6) overnight at 4°C. The plates were blocked with 1% BSA in PBS (130 mM NaCl, 4 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, 2.8 mM KCl, pH 7.4) for 60 min at room temperature. After washing with PBS–Tween (PBS with 1% Tween-20), 100 µl of cell lysate was added to the wells. The plates were incubated overnight at room temperature, and washed again in PBS–Tween. Detecting antibody (100 µl), peroxidase-labeled sheep anti-Human FXIIIA (Affinity Biologicals), diluted 1:500 (25 mM Hepes free acid, 25 mM NaCl, 1% BSA, 0.1% Tween-20, pH 7.2), was added to the wells and incubated at room temperature for 60 min. After washing, 100 µl *O*-phenylenediamine substrate (OPD, 10 mg in 24 ml in 13.5 mM citric acid, 25 mM $Na_2HPO_4$, pH 5.0) was added for 5–10 min until the color developed. The reaction was stopped with 2.5 M $H_2SO_4$. The plates were read in a BioTek ELx-800 microplate reader at 490 nm. A standard curve was generated using recombinant human FXIII protein (Neomarkers, Fremont, CA).

### RT-PCR

Total RNA from chondrocytes was isolated by the acidified guanidinium isothiocyanate method, using TRIzol (Life Technologies, Grand Island, NY). RNA was reverse-transcribed as previously described and the generated cDNA was amplified in a GeneAmp PCR System 2400™ thermocycler (Perkin–Elmer Biosystems, Foster City, CA) using a PLATINUM™ PCR SUPERMIX (Life Technologies). Published primer sequences for human types I and II deiodinases[18,19], FXIIIA[20] and type II Tgase[21] were used. Primer concentrations were 10 pm/µl. Reactions were run for 40 cycles at 56°C. Transcripts were analyzed in 1.6% agarose/TAE gel electrophoresis and visualized by SYBER GREEN-I (Sigma Chemical Co.) using the Alpha Imager™ 2000 system (AlphaInotech Co., San Leandro, CA).

### CYCLIC AMP ASSAY

Cyclic AMP (cAMP) levels were determined with a commercially available EIA kit used according to manufacturer's directions (Assay Designs, Inc., Ann Arbor, MI). Forskolin (7-deacetyl-7-[*O*-(*N*-methylpiperazino)-γ-butyryl] dihydrochloride, 25 μM; Calbiochem) and IBMX (3-isobutyl-1-methylxanthine, 25 μM; Calbiochem) served as positive controls to raise intracellular cAMP levels.

### ENDOPROTEINASE ASSAY

Chondrocytes were incubated with no additives or thyroid hormones for 4 h. Endoproteinase levels in media and cell lysate were measured with a SpecWorks™ Protease Assay Kit (Novagen, Madison, WI) according to manufacturer's directions. This kit measures activity of endoproteinases including trypsin, elastase, proteinase K, chymotrypsin, bromelain, thermolysin, pronase and papain.

### TOXICITY ASSAY

The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was used to assess the toxicity of culture additives[17].

### STATISTICAL ANALYSES

All experiments were repeated at least three times. A Student's *t*-test was used to determine statistically significant differences between groups. A *P* value less than 0.05 was considered statistically significant.

## 3. Results

### EFFECT OF THYROID HORMONES ON CHONDROCYTE TGASE ACTIVITY

As shown in Fig. 1, T3 and T4 each stimulate Tgase activity in a dose-dependent manner ($P<0.01$). Tgase activity levels increased from control values of 0.19±0.02 U/mg protein to 0.40±0.03 U/mg protein with 750 nM T4, and from 0.11±0.02 to 0.27±0.01 U/mg protein with 100 nM T3. Effects were maximal at 4 h of incubation, but were present as early as 1 h (Table I).

T4 produced a shift of Tgase activity from cell to matrix (Table II). Cell-associated Tgase activity was reduced from 39.1 to 19.4% with a concurrent increase in matrix-associated Tgase activity from 60.8% in control cell layers to 80.5% in T4-treated cell layers ($P<0.01$).

### COMPARISON OF T4- AND T3-INDUCED TGASE ACTIVITIES

Most cells possess thyroid deiodinases, which convert T4, the circulating form of thyroid hormone, to T3, the active ligand for thyroid nuclear receptors. As shown in Fig. 2, articular chondrocytes are capable of converting T4 to T3 at 4 h. Type II deiodinase was readily detected in articular chondrocytes by RT-PCR (Fig. 3), while no signal for type I deiodinase could be discerned (data not shown). When cellular effects require conversion of T4 to T3, a time lag in the effect of T4 compared with T3 is often seen. As shown in Table I, the time courses of effects of T4 and T3 on chondrocyte Tgase activity were similar. Taken along with the rapid onset of action of T4 and T3, these data suggest a non-genomic mechanism for thyroid hormone action.

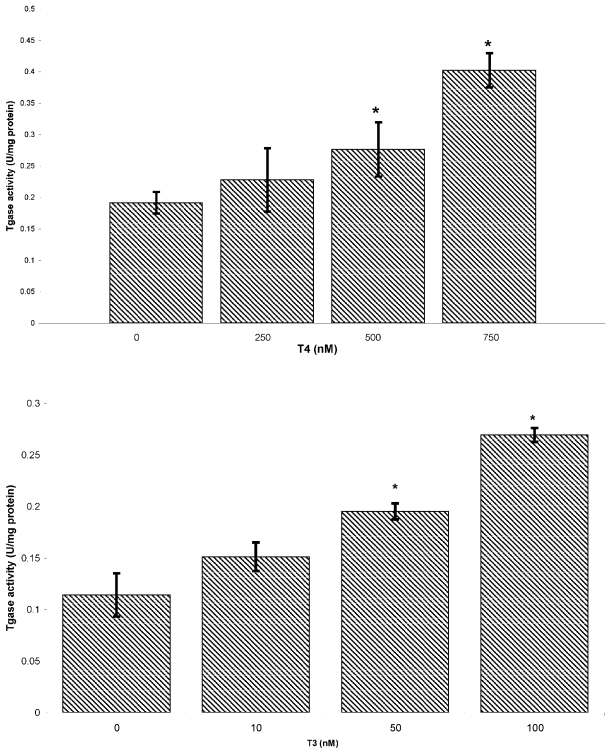


Fig. 1. The effect of T3 and T4 on chondrocyte Tgase activity. Chondrocytes were incubated for 4 h with no additives (control) or with 250–750 nM T4 (A) or 25–100 nM T3 (B). Tgase activity was measured in the whole cell layer and corrected for protein levels in the sample. Results are expressed as means±standard errors of the mean ($N=6$). *Reflects statistically significant differences from control values. T4 significantly increased Tgase activity at 500 nM ($P<0.02$) and 750 nM ($P<0.001$), while T3 increased Tgase activity at 50 nM ($P<0.008$) and 100 nM ($P<0.007$).

Table I
*Time course of effect of T3 and T4 on Tgase activity*

| Time | Tgase activity (% of control)* | |
|---|---|---|
| | T3 | T4 |
| 15 min | 129±7 | 112±4 |
| 30 min | 131±9 | 108±8 |
| 1 h | 174±10 | 170±14 |
| 2 h | 116±7 | 116±6 |
| 4 h | 140±20 | 255±30 |
| 24 h | 100±6 | 150±9 |

*Chondrocytes were incubated for various lengths of time with no additives (control), 100 nM T3 or 750 nM T4. Tgase activity was measured in the cell layer and expressed as a percent of control values. Values are means±standard deviations ($N=5$).

### EFFECT OF PROTEIN AND mRNA SYNTHESIS INHIBITORS ON T4-INDUCED TGASE ACTIVITY

To further investigate whether protein or mRNA synthesis was involved in this effect, we explored the actions of actinomycin and cycloheximide on the ability of T4 to induce Tgase activity. Actinomycin D had no effect on the ability of T4 to increase Tgase activity, suggesting that new mRNA synthesis was not necessary for this effect (Fig. 4). Actinomycin had a modest stimulatory effect on Tgase activity when used alone ($P<0.01$), suggesting that

Table II
*The effect of T4 on the percentage of Tgase activity in various cell compartments*

| | Tgase activity (% total)* | |
|---|---|---|
| | Cell-associated | Matrix |
| Control | 39.1±5.6 | 60.1±5.6 |
| T4 | 19.4±2.9 | 80.5±2.9 |

*Chondrocytes were incubated with or without 750 nM T4. Tgase activity was measured in the cell-associated and extracellular matrix compartments, and the percentage of activity in each compartment was calculated. Results are expressed as means±standard deviations ($N$=3). Tgase activity is higher in the matrix of T4-treated chondrocytes than in controls ($P$<0.01).

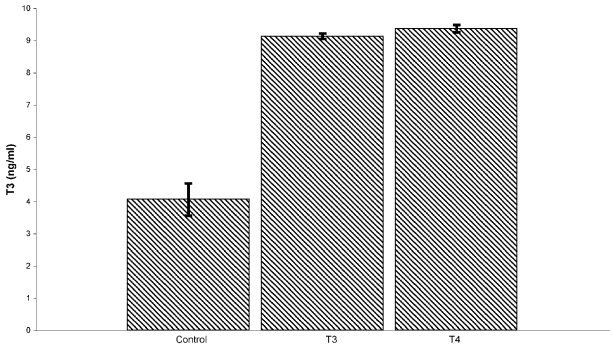


Fig. 2. The conversion of T4 to T3. Chondrocytes were incubated with no additives (control), 10 ng/ml T4 or 3 ng/ml T3 for 4 h. Levels of T3 were measured in the whole cell layer with a EIA kit. Results are expressed as means±standard errors of the mean ($N$=10).

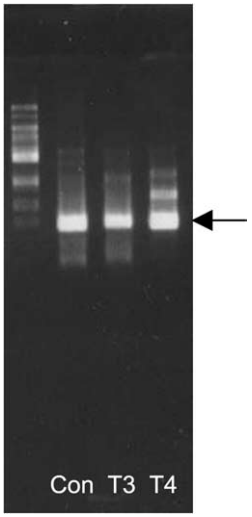


Fig. 3. RT-PCR of the type II deiodinase in chondrocytes. Total RNA was extracted from chondrocytes incubated with no additives (control), or 100 nM T3 or 750 nM T4 for 4 h. RT-PCR was performed using a primer sequence that recognizes human type II deiodinase. The arrow identifies a band corresponding to the expected molecular weight of the type II deiodinase sequence.

inhibition of mRNA production increased Tgase activity. Further evaluation of this effect is ongoing. The RT-PCR data confirmed only minimal changes in mRNA levels for the Tgase enzymes with thyroid hormone treatment.

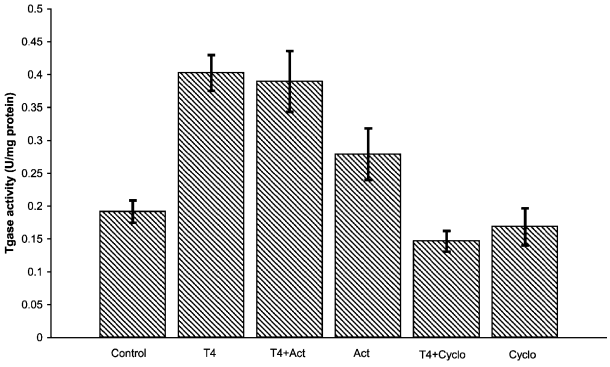


Fig. 4. The effect of protein and mRNA inhibitors on the ability of T4 to stimulate Tgase activity. Chondrocytes were incubated with no additives (control), 100 nM actinomycin (act), 500 nM cycloheximide (cyclo), or 750 nM T4 with or without 100 nM actinomycin or 500 nM cycloheximide for 4 h. Tgase activity was measured in the whole cell layer and corrected for protein levels in the sample. Results are expressed as means±standard errors of the mean ($N$=6). Actinomycin alone modestly increased Tgase activity ($P$<0.01). Cycloheximide suppressed T4-induced Tgase activity ($P$<0.001), while actinomycin had no effect on T4-induced Tgase activity ($P$>0.01).

mRNA levels for type II Tgase were slightly decreased by T4 or T3 [Fig. 5(A)], while mRNA levels for FXIIIA were slightly increased by T3 or T4 [Fig. 5(B)]. In contrast, cycloheximide inhibited the effect of T4 on Tgase activity and had no effect when present alone (Fig. 4). Tgase levels fell from 0.40±0.03 U/mg protein in T4-treated cell layers to 0.14±0.02 U/mg protein in cell layers treated with T4 and cycloheximide ($P$<0.001). Moderate increases in type II Tgase protein levels in T4-treated cultures were noted on Western blots (Fig. 6). FXIIIA levels as measured by Western blotting and ELISA were also modestly increased by T4 (Figs. 7 and 8). Similar results were seen with T3 (data not shown). Neither inhibitor was toxic. These data suggest that protein synthesis is necessary for thyroid hormone to stimulate Tgase activity.

### ROLE OF PROTEIN KINASE C OR cAMP IN THE EFFECT OF T4 ON CHONDROCYTE TGASE ACTIVITY

Protein kinase C and cAMP mediate some of the nongenomic effects of thyroid hormones in other settings[22,23]. The protein kinase C inhibitor, NGIC, suppressed the ability of T4 to stimulate Tgase activity ($P$<0.01), suggesting that activation of protein kinase C may be involved in this effect (Fig. 9). NGIC caused a modest increase in Tgase activity for unclear reasons ($P$<0.04), and was non-toxic. The protein kinase C dependence of this effect was confirmed by demonstrating translocation of protein kinase C activity from the cytosol to the membrane fraction of chondrocytes treated with T4 (data not shown). As shown in Fig. 10, levels of cAMP did not change in the presence of T4, although they were dramatically increased with exposure to the known cAMP stimulants forskolin and IBMX. There did not appear to be any correlation between cAMP levels and Tgase activity, as T4 was still capable of significantly increasing Tgase activity even in the presence of high cAMP levels.

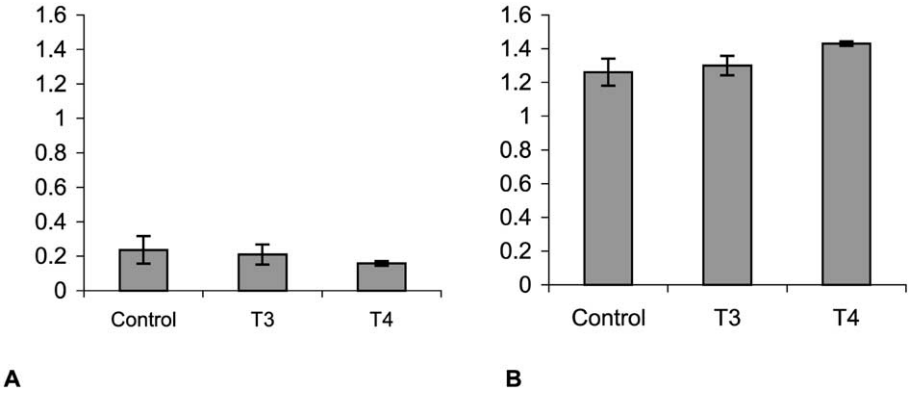


Fig. 5. RT-PCR of type II Tgase and FXIIIA mRNA. Chondrocytes were incubated with no additives (control), 100 nM T3 or 750 nM T4 for 4 h. Total RNA was extracted and RT-PCR was performed using primer sequences that recognize human type II Tgase, FXIIIA or the housekeeping gene GAPDH. Results are expressed as a densitometric ratio of (A) type II Tgase mRNA to GAPDH mRNA and (B) FXIIIA mRNA to GAPDH mRNA. There were no significant differences in quantities of type II Tgase or FXIIIA mRNA between control cultures and those treated with thyroid hormones.

EFFECT OF T4 ON ENDOPROTEINASE ACTIVITY

Certain proteases, such as trypsin and thrombin, stimulate Tgase activity through a variety of mechanisms[24], including direct proteolytic activation of FXIIIA[25]. As the effects of thyroid hormones on Tgase enzyme protein levels were so modest and did not correlate with the rapid effects on activity, we asked if T4 might affect Tgase activity by increasing endogenous proteinase activity. Using a kit that detects activity of trypsin and other endoproteinases, we demonstrated a significant increase in endoproteinase activity in the cell layer and media of T4-treated chondrocytes compared with control values (Table III). Endoproteinase activity levels were undetectable in untreated cell layers and media, and were 96±3 ng in the cell layer and 239±13 ng in the media of T4-treated chondrocytes. To further implicate endoproteinase secretion as a participant in the effect of T4 on Tgase activity, we examined the effects of the broad-spectrum endoproteinase inhibitor, α1 antitrypsin, on the ability of T4 to stimulate Tgase activity. The α1 antitrypsin (50 μg/ml) caused a significant decrease in T4-induced Tgase activity ($P<0.02$), while it had no effect on Tgase activity when present alone (Fig. 11). These data confirm a role for endoproteinase secretion in thyroid hormone-mediated Tgase stimulation.

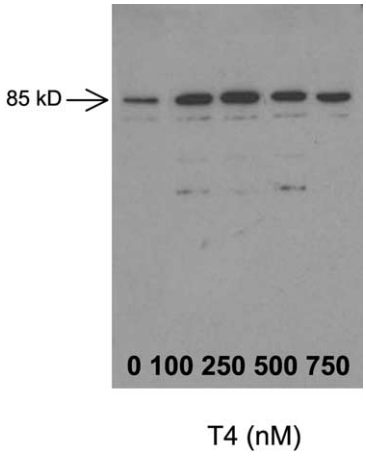


Fig. 6. Detection of type II Tgase by Western blotting. Chondrocytes were incubated with no additives or 100–750 nM T4 for 4 h. Lysed whole cell layer samples, corrected for protein content, were run on 10% Bis-Tris gels. After transfer to PVDF membranes, immunoreactive type II Tgase (85 kDa) was detected with an anti-type II Tgase antibody (Upstate Biotechnology, Lake Placid, NY), a peroxidase-linked secondary antibody and Supersignal®. Moderate increases were noted in type II Tgase protein levels in the presence of T4.

## Discussion

We report in this study that thyroid hormones increase Tgase activity in adult porcine articular chondrocytes. Similar concentrations of thyroid hormones stimulate other markers of the hypertrophic phenotype in articular chondrocytes, including type X collagen production, alkaline phosphatase activity and pyrophosphate production[13]. These data suggest that elevated Tgase activity is an additional feature defining the 'hypertrophic phenotype' in articular chondrocytes, and underscore the similarities between the aging chondrocytes responsible for pathologic

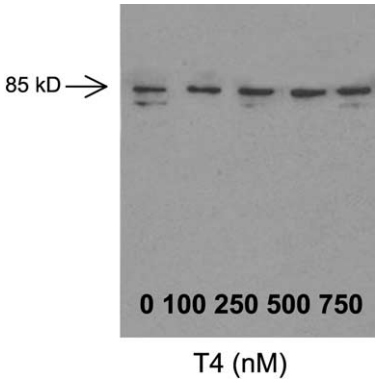


Fig. 7. Detection of FXIIIA by Western blotting. Chondrocytes were incubated with no additives or 100–750 nM T4 for 4 h. Lysed whole cell layer samples, corrected for protein content, were run on 10% Bis-Tris gels. After transfer to PVDF membranes, immunoreactive FXIIIA (85 kDa) was detected with an anti-FXIIIA antibody (Calbiochem), a peroxidase-linked secondary antibody and Supersignal®. Moderate increases were noted in FXIIIA protein levels in the presence of thyroid hormones.

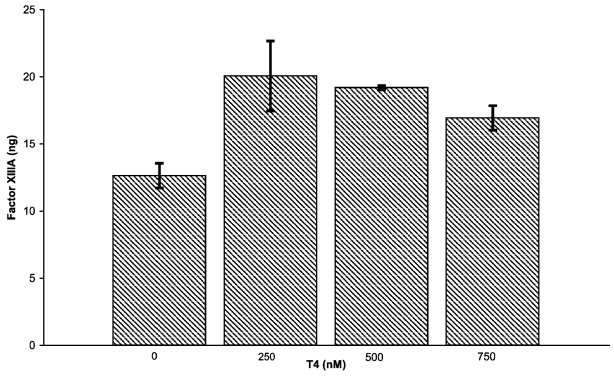


Fig. 8. Quantification of FXIIIA by ELISA. FXIIIA levels were measured by ELISA in the whole cell layer of chondrocytes incubated with or without T4 for 4 h. Results are expressed as means±standard errors of the mean ($N$=8). Small insignificant increases in FXIIIA levels were detectable by ELISA ($P$>0.05).

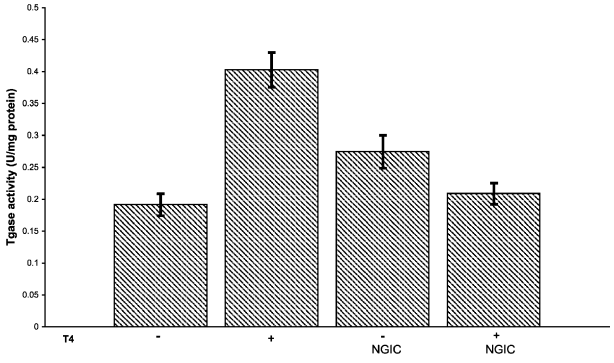


Fig. 9. The effect of protein kinase C inhibitors on the ability of T4 to stimulate Tgase activity. Chondrocytes were incubated with no additives (control), or with 750 nM T4 with and without the protein kinase C inhibitor, 10 nM NGIC, for 4 h. Tgase activity was measured in the whole cell layer. Results are expressed as means±standard errors of the mean ($N$=8). NGIC alone slightly increased Tgase activity ($P$<0.04). NGIC suppressed the ability of T4 to stimulate Tgase activity ($P$<0.01).

mineralization in articular cartilage and 'true' hypertrophic growth plate chondrocytes.

Although Tgase may play an important role in the matrix changes associated with degenerative arthritis[2,5], little is known about the regulation of the Tgase enzymes in articular cartilage. In other tissues, proteases, such as thrombin and trypsin, stimulate Tgase activity[7,8]. We show in this study that thyroid hormones increase endoproteinase activity. These proteases then may directly stimulate Tgase activity through a variety of mechanisms, including direct activation of the pro-Tgase form of FXIIIA[24,25]. Thyroid hormones also increase the percentage of Tgase activity in the matrix. It is the extracellular enzyme that likely participates in the matrix changes that facilitate pathologic matrix mineralization in degenerative arthritis.

The classic effects of thyroid hormones are mediated through nuclear receptors for T3, which on ligand binding, act directly on gene transcription. However, thyroid hormones also have well-described non-genomic effects. These are rapid effects that occur at the plasma membrane or in the cytosol and include effects on Na/H antiport[26] and adenylate cyclase activity[22]. The effects of thyroid hormone on Tgase activity in chondrocytes occur as early as 15 min after exposure. They are at least partially dependent on protein synthesis, but do not require new mRNA synthesis or conversion of T4 to T3. Altogether, these data support the hypothesis that stimulation of Tgase activity may be an additional non-genomic effect of thyroid hormone. The effect of thyroid hormone on Na/H antiport involves

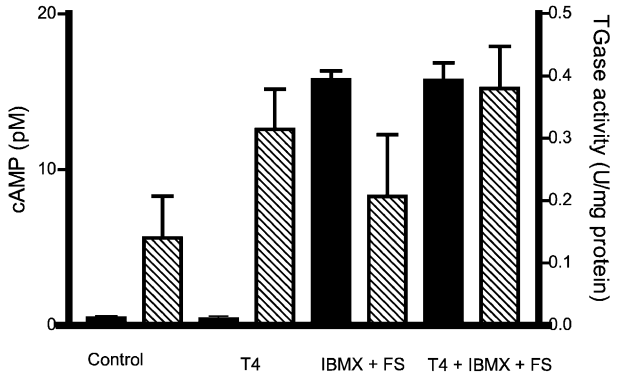


Fig. 10. The effect of T4 on cAMP levels in chondrocytes. Chondrocytes were incubated with no additives (control), or with 750 nM T4 with or without 25 μM forskolin (FS) and 500 μM IBMX. After 4 h, cAMP levels and Tgase activity were measured in the whole cell layer. Solid bars represent cAMP levels, while hatched bars represent Tgase activity levels. Results are expressed as means±standard errors of the mean ($N$=6). There were no differences in cAMP levels in the presence of T4 ($P$>0.01).

Table III
*The effect of T4 on endoproteinase secretion by chondrocytes*

| | Endoproteinase activity (ng)* | |
|---|---|---|
| | Cell layer | Media |
| Control | 0 (none detected) | 0 (none detected) |
| T4 | 96±3 | 239±13 |

*Chondrocytes were incubated with no additives (control) or with 750 nM T4 for 4 h. The endoproteinase activity in the cell layer and media was measured using the SpecWorks™ Protease Assay Kit (Novagen). Results are expressed as nanogram equivalents of a standard endoproteinase (Alcalase®), and are shown as means±standard deviations ($N$=3).

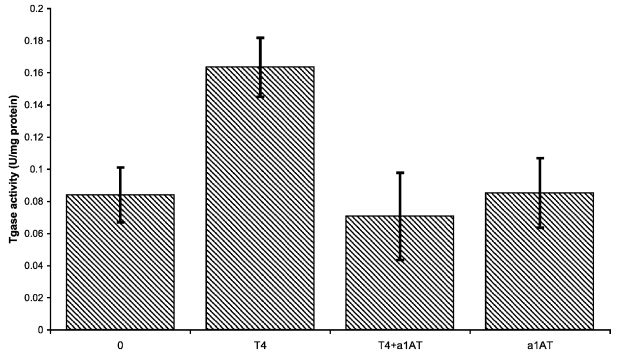


Fig. 11. The effect of the protease inhibitor α1 antitrypsin (a1AT) on T4-induced Tgase levels. Chondrocytes were incubated with and without 750 nM T4 for 4 h in the presence or absence of 50 μg/ml α1 antitrypsin. Tgase activity was measured in the cell layer and corrected for protein. Results are expressed as means± standard errors of the mean ($N$=6). α1 antitrypsin significantly suppressed T4-induced Tgase activity ($P$<0.02).

signaling through protein kinase C, and our work supports a similar protein kinase C-dependence.

The concentrations of thyroid hormones necessary to affect Tgase levels in articular chondrocytes are twofold higher than those measured in the circulation[27], and are slightly higher than those employed in other studies. There may be a different dose response for mature articular chondrocytes compared with younger chondrocytes or for non-genomic rather than nuclear-receptor mediated effects. We are not suggesting that T4 necessarily regulates Tgase activity in articular cartilage *in vivo*. There is no epidemiologic evidence that associates degenerative arthritis or calcium crystal deposition with hyperthyroid states. Conversely, remarkably little is known about local thyroid hormone metabolism in the joint. We demonstrate the presence of the type II deiodinase on articular chondrocytes, and show rapid conversion of T4 to T3 by chondrocytes. Serine proteases, such as elastase, which are present in synovial fluids in settings of inflammation and injury, cleave thyroid-binding globulins and dramatically increase local levels of free T4[28]. As T4 exposure also increases activity of endogenous proteinases, this may further increase local levels of thyroid hormone. Thyroid hormones have been shown to upregulate proteinase genes in other settings, such as during tadpole development[29].

In summary, we have shown in this study that thyroid hormones increase Tgase activity in adult articular chondrocytes through a probable non-genomic mechanism that is at least partially dependent on protein kinase C activity and involves an increase in the activity of endogenous endoproteinases. These findings support the hypothesis that Tgase is an additional component of the 'hypertrophic phenotype', and that factors that induce this phenotype likely contribute to pathologic mineralization in aging articular cartilage.

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